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'Giardia' Prevalence in Commercially Trapped Mammals

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16. ABSTRACT
Recent outbreaks of giardiasis have been linked to *Giardia* contamination of municipal water supplies. Human or animal contamination of drinking water has been implicated in most of the outbreaks. Of particular interest to water utility operators and water supply engineers is the possible animal contamination of water supplies which are protected from human contamination. A giardiasis outbreak in Camas, Wash., was thought to have resulted from aquatic mammal contamination of an otherwise protected water supply. Following this outbreak, the Washington State Health Services Division undertook a series of aquatic mammal surveys to estimate the prevalence of *Giardia* infection in Washington's wild beaver and muskrat population and to determine if animal contamination of other water supplies could occur. Many Washinton State water supplies use chlorination as the only treatment for surface water, and since chlorination may not inactivate *Giardia* cysts, there was concern that a number of cities were vulnerable to giardiasis outbreaks.

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Giardia Prevalence in Commercially Trapped Mammals

Floyd Frost, Byron Plan, Bill Liechty

Recent outbreaks of giardiasis have been linked to *Giardia* contamination of municipal water supplies (1,8,10). Human or animal contamination of drinking water has been implicated in most of the outbreaks (4). Of particular interest to water utility operators and water supply engineers is the possible animal contamination of water supplies which are protected from human contamination.

A giardiasis outbreak in Camas, Wash., was thought to have resulted from aquatic mammal contamination of an otherwise protected water supply (8). Following this outbreak, the Washington State Health Services Division undertook a series of aquatic mammal surveys to estimate the prevalence of *Giardia* infection in Washington's wild beaver and muskrat population and to determine if animal contamination of other water supplies could occur. Many Washington State water supplies use chlorination as the only treatment for surface water, and since chlorination may not inactivate *Giardia* cysts (3,6), there was concern that a number of cities were vulnerable to giardiasis outbreaks.

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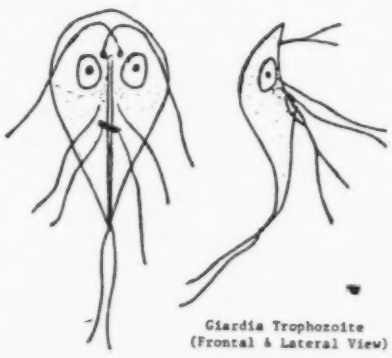
Background:

Giardia is a flagellated protozoan parasite which inhabits the small intestine of amphibians, birds and mammals (9). In Washington state it is the most commonly identified human intestinal parasite. Infection in humans can occur after ingesting as few as 10 cysts (11). Implicated modes of transmission are water, hand to mouth transfer, and possibly contaminated food (13). Recently, waterborne transmission has received much attention. Twenty waterborne outbreaks of human giardiasis were reported between 1971 and 1977, sixteen of which were associated with drinking untreated or minimally treated surface water (4). Outbreaks which affected a large number of people occurred in Rome, New York - 1975, Camas, Washington - 1976, and Berlin, New Hampshire - 1976. In each of these outbreaks, *Giardia* cysts were recovered from the municipal drinking water supply (4,8,10).

Giardia Cyst



Giardia Trophozoite

During the Rome outbreak, *Giardia* cysts were recovered for the first time from a municipal raw water supply. Patent *Giardia* infections developed in *Giardia*-free beagle puppies when fed sediment collected from the Rome water supply, suggesting that the waterborne cysts were infective for humans (4). During the Camas and Berlin outbreaks, cysts were recovered from the raw and treated water and from beaver (8,10). Three *Giardia*-infected beaver were trapped in the Camas watershed and one in the Berlin watershed. Attempts to infect beagle puppies with *Giardia* recovered from the Camas supply failed because cyst deterioration occurred prior to administration. No beagle studies were attempted during the Berlin outbreak. The *Giardia* contamination of the Camas water supply generated special interest because this watershed, unlike the Rome or Berlin watersheds, was uninhabited by humans and closed to public access. This finding suggested that beaver could function as reservoir hosts for *Giardia* and, thus, be of epidemiologic importance in human giardiasis. This has recently been supported in Colorado where two of three volunteers became infected after ingesting beaver *Giardia* (5).

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Following the Camas outbreak, statewide surveys of aquatic mammals were begun to determine the extent of *Giardia* infection among Washington's aquatic mammals. Much of the drinking water in Washington originates from surface sources. Furthermore, many cities which obtain drinking water from streams use chlorine as the only method of treatment, provided that strict watershed protection and low turbidity levels can be maintained. Since chlorine may not be effective in inactivating cysts (3,6), and these watersheds often contain good habitats for beaver and other aquatic mammals, the risk of human infection through contaminated drinking water may be significant.

Materials and Methods:

Commercial trappers were recruited to provide stool samples from kill-trapped animals. Those who agreed to participate were supplied with sampling kits containing vials, mailing containers, instructions on how the sample was to be obtained, and a survey form. The first year of the survey the vials contained no fluid. During the second and third years the vials contained 2.5% formalin solution. The survey form requested information on the animal species, sex, age, date sample was taken, and location where the animal was trapped (watershed, township/range/section, county, nearest town). During the third year of the survey, information on the general health, fur, body fat and injuries to the animal was also requested. The trappers collected the samples from the large intestine or rectum of the animal using a wooden stick included with each kit. The sample was then placed in the vial and mailed to the State Public Health Laboratory. Only one sample was taken per animal. Time between trapping and receipt in the lab was generally less than ten days.

Trappers were encouraged to submit samples from animals trapped in municipal watersheds. Special arrangements were made for obtaining animals from protected watersheds. During the first year, only beaver samples were requested. Beaver, muskrat, mink, racoon and river otter samples were requested during the second and third years.

Once the stool samples were received at the State Public Health Laboratory, they were processed by the formalin-ether technique (12). This procedure was selected because it permitted the processing of large numbers of samples without requiring immediate analysis.

After a stool sample was processed, the sediment was thoroughly mixed in 1 ml of 2.5% formalin. An aliquot of this mixture was immediately removed and placed on a microscope slide, at which time it was mixed with a drop of Lugols iodine and covered with a 22mm x 22mm coverslip. The entire coverslip was examined at 100x using a compound microscope. Structures resembling *Giardia* cysts or other parasites were verified at 450x. All parasites and ova seen were identified to genus and recorded during the third year. Only *Giardia* was recorded during the first two years.

Parasite abundance for each species of protozoan was estimated by counting the number of organisms encountered on five different 100x fields, averaged and multiplied by 169, the number of 100x fields seen on a 22mm x 22mm coverslip. In each sample, a given quantity, approximating one gram of feces, was processed. Statistical analysis was done using SPSS t-test and a logit analysis program.

Results

During the three years of the study, 704 fecal samples were examined. Parasites were found in all species of animals from which samples were submitted; however, only beaver and muskrat harbored *Giardia* (Table I). The percentage of *Giardia*-positive animals increased each year, due to the detection of infected animals in previously negative counties and to a higher prevalence of *Giardia* infections in animals from previously positive counties.

Giardia prevalence in muskrats was higher than in beave for the second and third years ($p < .01$). Juvenile beaver were more often found to be infected than were adults ($p < .01$) during both the '77-'78 and the '78-'79 trapping seasons. Muskrat samples pooled for the '77-'79 trapping seasons also showed a higher positivity among juveniles ($p < .05$).

When cyst density measures were compared, the number of cysts per coverslip was greater for beaver than for muskrat ($p < .05$). This difference was due to many beaver and no muskrat having extremely high cyst counts. Cyst density comparisons for adults versus juveniles of the same speices did not demonstrate significant differences between age groups.

No significant differences were detected in the prevalence of other parasitic infections among

Table 1
Parasite Findings

Number trapped (Percent *Giardia* infected)

Animal	Parasites	1976/1977	1977/1978	1978/1979
Beaver	G,C,Tr	173 (6.3%)	177 (6.8%)	179 (19%)
Muskrat	G,E,Ch,C, Tr,N,Tc	1	17 (35.2%)	115 (42.6%)
Nutria	C,Tr	1	2	5
Mink	Tr,N,Tc		5	7
Raccoon	C,N,Tc		4	24
River Otter	Tr	1	2	10
Bobcat	To			7
Coyote	To			2
Lynx	To			1

G= *Giardia* sp. Ch= *Chilomastix* sp. To= *Toxocara* sp.
C= coccidia Tr= trematode eggs Tc= *Trichuris* sp.
E= *Entamoeba muris* N= nematode larvae

Table 2

Parasite Associations of Beaver and Muskrat

Beaver	*Giardia* Positive (34 animals)	*Giardia* Negative (145 animals)
	67.6% no other parasites	57.9% no other parasites
	11.8% coccidia	19.3% coccidia
	11.8% trematode	15.9% trematode
	8.8% coccidia, trematode	6.9% coccidia, trematode
Muskrat	*Giardia* Positive (49 animals)	*Giardia* Negative (66 animals)
	18.4% no other parasites	45.5% no other parasites
	22.4% *E. muris*	10.6% *E. muris*
	14.3% trematode	22.7% trematode
	22.4% *E. muris* and other parasites	13.6% *E. muris* and other parasites
	22.4% other trematode, coccidia, nematode, *Chilomastix* combinations	7.5% other trematode, coccidia, nematode, *Chilomastix* combinations

Table 3

Beaver and Muskrat Positivity By Month Trapped For 1978/79 Survey

	Beaver		Muskrat	
Month	Examined	Percent *Giardia* Pos.	Examined	Percent *Giardia* Pos.
Nov.	81	23.4	56	53.6
Dec.	78	16.7	34	38.2
Jan.-Oct.	20	10.0	25	24.0

Table 4

Animal Samples by Geographic Region

Season	Results	Region			
		West	Northwest	Southwest	East
76-77	POS.	2	1	6	2
	NEG.	31	43	23	65
77-78	POS.	3	3	5	1
	NEG.	28	72	19	47
78-79	POS.	7	13	7	7
	NEG.	30	60	14	41

COUNTIES

WEST: Clallam, Grays Harbor, Kitsap, Mason, Pacific, Jefferson
EAST: Chelan, Columbia, Ferry, Garfield, Grant, Kittitas, Lincoln, Okanogan, Pend Oreille, Spokane, Stevens, Walla Walla, Yakima, Adams, Asotin
NORTHWEST: King, Pierce, Skagit, Snohomish, Whatcom
SOUTHWEST: Clark, Cowlitz, Lewis, Skamania, Thurston

LOGIT ANALYSIS FACTOR	G^2	DEGREES OF FREEDOM
Year	16.2	2 $p < .005$
Region given year	18.9	3 $p < .005$
Residual	3.1	

Giardia-positive versus *Giardia*-negative beaver. Differences were observed for *Giardia*-positive versus *Giardia*-negative muskrat in the prevalence of other infections ($p < .01$). A higher percentage of *Giardia*-negative muskrat had no other parasitic infection and relatively few had multiple parasite infections (Table II).

Most samples were received during the trapping season (November through February), which limited the examination of seasonality of *Giardia* cyst excretion to these months. A downward trend in *Giardia* positivity was observed for both beaver and muskrat, by month trapped ($p < .01$), with November having the highest positivity (Table III). The period January to October primarily contains animals trapped in January or February. Only rarely were animal samples received after March 1.

Sex of the animal was not shown to be related to risk of *Giardia* infection for either beaver or muskrat. It should be noted that age and sex determinations were made by the trappers. It is questionable whether accurate sex determinations were made for beaver (7).

The prevalences of *Giardia* in beaver were compared by geographic area. Muskrat were not included in the analysis because of the small sample size and higher prevalence of positive stools. The state was divided by counties into geographic divisions: western counties bordering the ocean, northwest counties, southwest counties and counties east of the Cascade Mountains. Logit analysis suggests that positivity was not uniform over these regions ($p < .01$) (Table IV). Beaver from southwest and western counties showed a higher prevalence of infection than did beaver from Cascade or eastern counties.

Differences in prevalence were also examined by level of protection of the watershed. Three groups were considered: 1) the Green and Cedar River watersheds, 2) other protected watersheds, and 3) areas outside of protected watersheds. Logit analysis revealed no differences in prevalence for these three areas during the three years of the study (Table V).

Table 5

***Giardia* Positivity by Type of Area**

Season	Results	Watershed		
		Cedar and Green River	Other Restricted Watersheds	Other
76-77	POS.	0	1	10
	NEG.	19	9	134
77-78	POS.	0	1	11
	NEG.	16	12	137
78-79	POS.	7	2	25
	NEG.	22	7	116

LOGIT ANALYSIS FACTOR	G^2	DEGREES OF FREEDOM
Year	17.9	2 $p < .005$
Watershed	.3	2 $p < .1$
Residual	5.5	4

Discussion

The investigation has determined that *Giardia* is a common intestinal parasite of beaver and muskrat in Washington. The study should not be considered a random sample of beaver and muskrat because the manner in which samples were obtained did not give equal probability of inclusion for all animals in the state. If cyst passage for these mammals is similar to human cyst passage, the use of a single stool sample per animal may bias the results by underestimating the true prevalence of infection. For these reasons the prevalence estimates may not indicate the true prevalence of infection in aquatic mammals but may be regarded as an approximate minimum prevalence level. Nevertheless, the study documents a widespread distribution of infection across the state and indicates that the potential for water contamination by aquatic mammals exists throughout Washington.

The increased prevalence of *Giardia* in beaver and muskrat observed each year may have been the result of true increases in *Giardia* positivity or the result of better or different methods of parasite detection. As stated earlier, sample collection methods changed slightly between the first and second years. Also, the precise locations where animals were trapped varied from year to year, which may have contributed to changes in prevalence. However, logit analysis revealed that trapping season was significant even after adjusting for differences in prevalence by region.

Some researchers have suggested that sewage plays an important role in maintaining infection of aquatic mammals (5). The results of this study and the Camas outbreak investigation (8) do not support this contention. The finding of infected animals in the Jones Creek/Boulder Creek, Cedar River and Green River watersheds suggests that beaver can maintain the infection independent of human involvement. The possibility that infected beaver migrated from outside the watersheds (areas less protected) cannot be ruled out, but the finding of infected beaver at four different locations within the Cedar and Green River watersheds, three of which were separated from each other by at least nine miles, suggests that this was unlikely. Transmission from a single infected beaver to other beaver is a possibility, but positive beaver were found in locations fed by separate drainages.

Beaver and muskrat were the only animals identified as *Giardia* infected. Previous authors (2,5) report *Giardia* infected beaver and muskrat. However, no reports are available on how commonly wild animals are infected or what host or environmental characteristics predispose wild animals to infection. This study found that host age was related to finding cysts in the stool, as juvenile beaver were found positive 3.9 times more often. Whether adults become free of the infection, develop latent infections or shed cysts in lower or more variable levels cannot be answered from these data. The density of cysts in the stools presents a somewhat more confusing picture. Beaver show a large variance of cyst densities but a low prevalence of infection. For muskrats this is reversed.

Implications of the geographic distribution analysis is unclear. Whether the higher prevalence in west and southwest Washington (Table VI) is related to animal abundance, to a closer relationship to human populations, or to other factors cannot be answered. However, the finding of infected animals in well-protected watersheds suggests that human sewage is not necessary to maintain infections among wild beaver. It also suggests that protected watersheds will not insure pathogen-free water.

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Our Cover . . .

Solar-Powered System Monitors N.J. Air

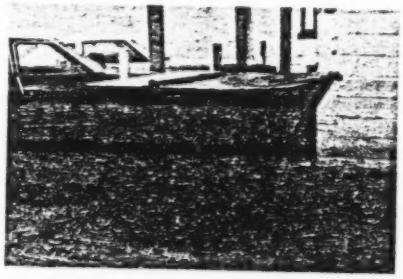
The nation's first solar-powered air monitoring station recently went into operation in Liberty State Park, N.J. The station cost $93,000, and was jointly funded by the Department of Energy and the New Jersey Department of Environmental Protection.

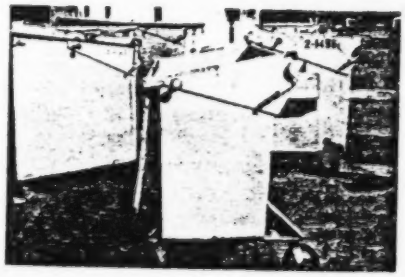
The 360-watt system uses 860 individual silicon solar cells, set into 20 photovoltaic modules, to collect and convert energy from the sun. The 96-square-foot array is mounted atop a 17-foot tall frame. Excess electrical energy produced by the system is stored in a bank of specially designed, lead acid batteries, and is used to run the station during the evening and early morning.

The station monitors the air every sixth day for a period of 24 hours. It was designed by NASA's Lewis Research Center.

Refuse Bins Must Meet Stability Need

The U. S. Consumer Product Safety Commission is cracking down on unsafe refuse bins that can injure, or even kill, children who play around them. Unstable bins which can easily be tipped over by children have been banned by the federal government since June, 1978, but a preliminary survey of garbage bins by 40 states and some local governments indicates that approximately 38 percent of bins subject to the ban may be in violation. To date, CPSC has filed over 250 noncompliance cases against refuse haulers who were renting, leasing, or providing unstable bins as part of a refuse service. The Commission has obtained an additional 40 test kits to help extend surveillance of the refuse collection industry, and encourages state and local authorities to help, either voluntarily or under contract, to enforce the ban of unstable bins. The ban generally requires that empty metal garbage bins of 1 cubic yard or greater not tip over when either a 191 pound vertical force or 70 pound horizontal force is applied on the most unstable edge. Since the ban, CPSC has learned of four deaths and several serious injuries to children between the ages of 4 and 7 due to garbage bin tipover. — Ross Koeser





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